Notes on Some Botanical Reading Done in the Laboratory of Prof. Schwendener in Berlin, in June and July, 1889.*

By Emily L. Gregory, Ph. D.

This botanical reading, as it is termed in the heading of my paper, was not undertaken with an object of studying any one line of work, but rather with that of acquainting myself with the principal botanical work which has occupied the northern botanists of Germany since the year 1886, and more especially during the latter part of that time.

One of the first subjects taken up was that of Mycorhiza. A point of interest for me in this was the summary manner in which Prof. Schwendener disposes of the question of symbiosis among plants. One of the first elaborate papers on the Mycorhiza is that of Prof. Frank, published in the "Berichte der deutschen botanischen Gesellschaft" in 1885. This publication called forth comments from several authors claiming priority of discovery. Prof. Woronin claims that the principal facts given in this paper of Frank's were published some time previous by Herr Kamienski, in an an article on *Hypopitys Monotropa*. Rees has an article in the same journal in which he says a fungus, very similar to the one described by Frank, was discovered and named by him, *Elaphomyces*. However this may be, it is beyond controversy that Frank is the first one to give a detailed account of this singular growth. He defines the term Mycorhiza as the "peculiar organ of the root of the family Cupuliferæ and some other plants, which organ is formed by a union of fungus hyphæ and the root itself." The other plants referred to here are some of the Conifers and a few of the Ericaceæ.

As is very well known, the organ of the plant for obtaining nourishment from the soil is the root hair. These hairs occur at a short distance back of the growing tip of the rootlets, forming a little zone of varying width around the young root. They die off as the root extends itself in length, new ones springing out near the tip, so that these real food organs of the plant are constantly being renewed as the young rootlet grows in length only from the tip. In those plants where the Mycorhiza

* Abstract of a paper read before the Club, Oct. 23d.

occurs no such root hairs develop. We are not at liberty to infer from this that the plant has lost the ability to produce them, because what occurs after the germination of the seed would prevent the young epidermal cells of the root from extending themselves into hairs after the normal method.

What happens really is this: Shortly after the development of the little roots, and before the time for the root hairs, a fungus hypha attaches itself to the young root not far from the tip. This hypha thread, after fixing itself firmly to the root, grows with great rapidity, branches and rebranches, winding itself about the root and forming a complete network around it. As it increases in age it forms a so-called pseudo-parenchymatic tissue, by means of numerous partition walls. This new tissue, encasing the root, is small celled, thin walled and smooth on its surface, except that hyphæ are sent out from various parts which extend into the soil, fasten themselves upon the little particles in exactly the same manner as the real root hair. As the growing tip of the root prolongs itself, thus developing a new, fresh surface not covered by the fungus, new branches grow out from the parts nearest the tip, and after a time grow fast to this as in the older root. So there is always a little part of the growing root tip free from the fungus, and always a little zone around which the fungus is coiling, but has yet no genetic connection with the root.

Back from this zone the fungus hyphæ are grown fast to the epidermal cells, penetrating the walls and entering into the side walls of these cells, but never into their lumen. As Frank describes it, the fungus hyphæ are grown fast to the tissues of the root just as the hyphæ of the lichen are grown fast to the Algæ. This combination of fungus hyphæ and root has been named Mycorhiza or fungus root. This curious instance of parasitism, or whatever name may be given it, has excited no little discussion on the part of the professors forming the society known as the Deutschen Botanischen Gesellschaft, at Berlin. Frank, who is professor in the Landwirthschaftliche Hochschule, or agricultural college, is inclined to take the extreme view, and actually goes so far as to claim that the lofty tree, with its sweeping branches laden with innumerable little work shops for the manufacture of

carbo-hydrates, is, after all, nothing but a parasite on the microscopic fungus hidden in the ground below. That this is an extreme view may easily be recognized when the real facts, which are known to be facts, are taken into consideration. That the plant has no other means of getting nourishment from the ground than through these hyphæ is a known fact. But on the contrary, nothing is known of the true nature of this food when given over to the plant. If it were proven that the fungus takes up elaborated food from the humus in which it is found, and passes that food over to the tree, it could not then be considered the host from which the tree gets its living, but only the conductor of food. It is conceded by even the most conservative that the tree may obtain some elaborated or energized food in this way; but the real idea of parasitism includes a greater dependence of the parasite upon its host, or rather a different kind of dependence than this, namely, a plant living upon another, by taking the food which is elaborated by this plant for its own purposes.

Now, in the Mycorhiza it is not known even that the tree receives food drawn from the humus through the hyphæ. That this is presumably the case adds nothing to substantiate Prof. Frank's statement that the beech tree is a parasite.

In regard to the point about the lack of the root hair, the statement made is correct so far as actual experiments have been tried. Yet it would not be at all surprising if an experiment should show that the plant had lost its power of producing root hairs. The general law of inheritance is, that a plant possessing an organ whose function is usurped by that of some other organ, extraneous or otherwise, in the course of generations loses its power to develop this organ. There is no question but that some very interesting biological facts are yet to be discovered in this field. The relationship between plants of different groups, which has been conveniently named Symbiosis, has been proven to be of so varied a nature that the word itself has scarcely any definite meaning. In the earlier history of this word, in its application to botany, some botanists tried to limit its use to that relationship by which two plants of different groups are genetically connected with each other, neither being injured by this

connection, and either one or both receiving therefrom a positive gain. The difficulty here seems to be that it is impossible to say whether an injury is done to the plant or not. Prof. Schwendener, in discussing the question, gave for an example the lichen. Some botanists assert that the presence of the fungus, instead of retarding the development of the alga enclosed, is conducive to its growth and development. This statement, he claims, is not substantiated by fact. Who can say that it is not an injury to the alga that it has lost its power of sexual reproduction? It is quite true that while it has lost in this way, it has gained in ability of vegetative propagation. It is a point which no scientist has yet been able to determine, that is, to exactly balance the gains and losses so that it is possible to say whether the alga would have reached a higher stage of development had it not been made use of by the fungus. Prof. Schwendener summarily disposes of this question by declaring that the word Symbiosis may be applied to any combination of individuals for mutual benefit.

Another question, and one including a far wider range than this, goes back practically to the old one, "do plants make use of the free nitrogen of the air?"

This old question comes up in new forms, and like many other old questions seems only to increase in difficulty as it increases in age.

In a certain form of this subject Frank also plays a very prominent role as investigator. A word here in explanation of the fact of so many subjects taken up him.

He holds a prominent position in this agricultural college, and the question of adaptation of crops to soil in Germany is one of great practical importance. Therefore the authorities, or those in charge of this institution, claim from the professors engaged in it, a certain amount of work in practical directions. This has led Prof. Frank to take up questions bearing decidedly on the chemical side of plant physiology. As he is not himself a practical chemist, he employs one in his laboratory to make his most important and finest chemical tests.

In respect to the form in which this question is now being worked over in Berlin, there is one branch of it which seems to

me may be of interest, though, of course, the subject is too broad to be treated only in the most general way.

This branch is included in the peculiar characteristic of the Leguminosæ, which renders them able to produce more nitrogen than plants of other families. I may be excused here for bringing together a few statements probably familiar to you, but which will serve to make clear the relation of this question to the general one, as to the source of nitrogen for the plant.

In nearly all text books in physiological botany, it is claimed that green plants get their nitrogen chiefly from the soil in the form of nitrates. The principal authority on which this rests is the experiments of Boussingault, made forty years ago. These experiments have never been refuted, although many other scientists have reached conclusions directly opposite to those of Boussingoult. For example, Ville, in Paris, claims to have proven that certain plants do take part of their nitrogen supply from the free nitrogen of the air. Lawes and Gilbert, of England, on the other hand, claim that the evidence given of this is not sufficient, in short, they claim to have disproved the conclusions reached by Ville. Many others have experimented in this field, but among the conflicting opinions we may select one or two that have been decided by general consent to rank as facts. These are, 1st, that the plants of the family Gramineæ obtain their nitrogen mostly from the soil, and that the amount of nitrogen produced by these plants is in direct proportion to that of the soil where they grow. Second, plants of the family Leguminosæ, without regard to the amount of nitrogen in the soil in which they grow, produce two or three times as much in their fruit as do the family Gramineæ. These facts are so far recognized, that the Leguminosæ are called nitrogen producers, the Gramineæ, especially the cereals, nitrogen consumers.

This brings us to the subject at once, with the question: If there is no direct relation between the amount of nitrogen in the soil and that found to be produced by the plant, where does the plant get it? If from the air, is it in the form of free nitrogen or as ammonia?

Many of the family Leguminosæ are supplied with peculiar

tubers on their roots, and it seems quite well authenticated that there is some connection between these tubers and the production of nitrogen. Under the direction of Prof. Frank, Brunchoist took up the study of these tubers. He says of their history they have been considered everything possible for such a part of a plant to be, viz.: insect-galls, sclerotia, lenticels, vegetative buds, (which under certain circumstances might grow out,) fungus-galls and finally as albumin builders and reserve holders. This latter view is the one supported by Brunchoist, based upon the result of his experiments and study under Frank. He considers them as normal outgrowths of the plants. Woronin, who studied these bodies previous to the work done by Brunchoist, discovered that in the parenchymatic cells of these tubers were little bodies resembling bacteria. Also in certain cells he found fungus hyphæ, but never in the same cell containing the bacteria-like bodies.

Now Brunchoist claims that he has discovered the origin of these little bodies, that they develope out of the living protoplasm of these cells and are not bacteria, but gives them the name bacteroiden, or bacteria–like bodies, having the form but not the function of bacteria. He claims that their function is to supply the plant with nitrogen, later detected in its nitrogen-holding fruit, and that they dissolve and are passed upward in the sap-current to the upper part of the plant.

While these investigations have been going on in Berlin other botanists have by no means remained idle. Hellriegel, who is well known in this controversy, takes a position opposing Frank, and contrary to the conclusions of Brunchoist.

In the Berichte of June 25th is an article by Frank, in which he gives about the sum of Hellriegel's opinions as follows: Hellriegel claims to have proven by his experiments that the Leguminosæ find in the free nitrogen of the air a source of food which the Gramineæ cannot make use of. The Leguminosæ have not however, of themselves, the power to assimilate this free nitrogen, but that they depend on the aid of certain "Mikro-organismen" to accomplish this act of assimilation for them, and that these organisms live in a *symbiotic* relation with these leguminous plants.

In other words, Hellriegel believes that these little bodies are really bacteria, and that they have the power to make use of the nitrogen, that they work it over into a form which the plant can use, and it is appropriated by these tuber-producing plants.

On the other hand, Frank holds that the experiments of Hellriegel do not bear out this conclusion, that the ability of plants to assimilate free nitrogen is much more extended than the experiments of Hellriegel lead us to suppose. He has nowhere given so clear and succinct a statement of his conclusions as Hellriegel, but he claims that the fact of the Leguminosæ producing more nitrogen than any other plants proves nothing in reference to their need of any special organ for this purpose. He lays much stress on the fact that at certain periods of their life they do not assimilate more nitrogen than other plants; for example, before blossoming they assimilate much slower than after that time up to the ripening of their fruit. This he gives as a possible solution of the Boussingault experiments, namely, that they were made at such a time in the development of the plants when they were not using much nitrogen. Again he argues: it is admitted that some plants have a greater power of assimilating CO_2 than others; why not also a difference in regard to nitrogen? Furthermore, he claims to have proven that plants belonging to other families than the Leguminosæ, as certain Cruciferæ, Gramineæ and some Algæ, in none of which these tubers occur, have yet the power to use free nitrogen of the air. He says that it has not been proven that these bacteroidea are able to assimilate nitrogen, but that this is known to be the part of the green plant.

Frank appears opposed to nearly all the botanists now at work at this question and those involved in it, in these two particulars. He holds that more plants assimilate free nitrogen than those having these peculiar tubers and belonging to the family Leguminosæ, and also that these little bodies, discoved by Woronin, are the normal product of the plant, and have no connection whatever with any outside organism.

Now the next step in this interesting question follows very naturally. This is: Do bacteria act in the process of changing the ammonia of the air into nitrates? This question, like many other physiological ones, has long been the study of chemists. Once

proven that this is the case, that bacteria are the means by which some of the inorganic material of plant food is rendered fit for their use, and we have another important link in the chain which binds together all living organisms. This would also go far to substantiate the theory that the tubers of the Leguminosæ are caused by bacteria extraneous to the plant itself.

One side point in connection with the tubers of Leguminosæ may be of interest. In a number of the Berichte of 1887 is a long article on this subject by Tschirch, also a member of this agricultural college in Berlin. In this article he speaks of the notion of Brunchoist that these bacteria-like bodies originate directly from the protoplasm of the cell, and adds that this idea was also maintained by Prof. Wigand, of Marburg, in a paper, not at that time published, on "The origin of bacteria in the closed plant tissues of the root tubers of Papilionaceæ." He also adds that although Prof. Wigand agreed with Brunchoist as to the origin of these bodies, he differed widely from all other scientists in the construction he put upon this supposed fact. It so happened that I was passing through Marburg at the time Prof. Wigand was engaged in this study, and, at his request, I remained over one day to watch the development of these supposed bacteria in certain root tubers then being studied by him. The plant was *Adoxa Moschatellina.* Since that time his unfinished papers have been published, in which he gives a full account of these and other experiments on which he bases his theory of Anamorphism. He was always very careful to distinguish between this theory and that of "spontaneous generation" of earlier times. He claimed that the active protoplasm of the cell underwent some change, by which it passed over into these little bodies which he called bacteria.

The weak points in his and all the succeeding experiments made in this direction appear to be these: It is impossible to determine with certainty, first, that these bodies really are bacteria; second, if they are, what is their exact manner of origin. As long as these two points are not satisfactorily known, any theory based upon their presence, of course, can have no positive scientific value.